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ARTICLES FROM DOKLADY AKADEMII NAUK (BIOPHYSICS)

-USSR-

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Contents

PROTECTIVE EFFECT OF PHYTOLIPOPOLYSACCHARIDES AND VB-2 AGAINST IRRADIATION

[Following is the translation of an article by V. A. Semdak, Ye. P. Grachova, B. N. Gladyshev, and V. I. Sualikov in the Russian-language periodical Doklady Akademii Nauk (Reports of the Academy of Sciences USSR), No 4, Moscow, 1962, pp 925-928.]

(Presented by Academician A.I. Oparin 13 April 1962)

We know that the bacterial lipopolysaccharides (BLP) have a pronounced effect on the functioning of human and animal bone marrow. This is manifested in heightened leucocytosis (with an intensification of the phagocytic activity of the leucocytes and reticulo-endothelial cells), which apparently strengthens the resistance of man and animals to infection (the BLPs do not affect erythro-thrombopoiesis) (refs 1-9). However, the high toxicity of the BLPs and their ability to stimulate fibrinolysis hinder the use of these compounds as protective agents against penetrating radiation.

For this reason, from the standpoint of research on defense against penetrating radiation, the compounds of the lipopolysaccharide type found by B.N. Gladyshev (Biochemistry Institute of the Academy of Sciences USSR) in the higher plants (phytolipopolysaccharides -- PLP) (refs 10, 11) could be of some interest. The existence of a chemostructural similarity between the BLPs and PLPs led to the supposition that the PLPs might also exhibit biological activity, including that of stimulating in some degree the functioning of bone marrow. We also surmised, bearing in mind the origin of the PLPs, that the latter lack or possess very low toxicity.

The present study, a continuation of the search for protective agents against penetrating radiation (ref 12), involved tests of a preparation made from tea leaves which does not exhibit a fibrinolytic effect (oral presentation by G.V. Andreyenko, Moscow University). The intestinal mucosa was protected from radiation (refs 13, 14) and exhaustive diarrhea prevented by means of the VB-2 preparation which is a vinylbutyl ether polymer (mol wt 6000; n_D^{20} 1.4600; viscosity in 10% benzene solution 6-10 centistokes).

Method. The study was carried out on white male rats with normal hemograms. Both the test and control specimens were subjected to total irradiation with γ-rays from a Co^{60} source (700 r dose) under the following technical conditions: DSM-49, dose rate 62 r/min. For uniform γ-irradiation, the rats were treated in groups of 5-10. The animals in group I received four subcutaneous injections of PLP each in the pelvic area for a total of 200-400γ per rat weighing 200 grams, with each injection containing 100-50γ of sterile solution of the agent (sterilization in autoclave): 1st injection 96 hours, 2nd injection 72 hours, 3rd -- 48 hours, 4th -- 24 hours prior to irradiation. The specimens in group II were tested for combined effect of PLP and VB-2 preparation: the latter was administered perorally 24 hours after irradiation and then daily throughout the entire observation period (30 days). The animals in group III were used for control. Peripheral blood was studied for all animals. Determinations were made of the hemoglobin, erythrocytes, leucocytes and leucocyte formula, reticulocytes, and thrombocytes. The state of the blood was studied prior to irradiation and after various time intervals (1; 5; 10; 15; 20 and 30 days) upon irradiation. The data were processed statistically by V.I. Suslov on the M-2 computer.

Results. The study of the protective effects of the PLP and VB-2 preparation revealed that with the administration of the PLP preparation 24 hours in advance of irradiation, the death rate among the animals was 26%. With combined protection the rate fell to 23%. In the control group, the corresponding rate was 62%.

Table 1

The Effect of PLP and VB-2 Preparations on the Survival Rate of Rats Irradiated With a 700 r Dose From a Co^{60} Source

Вариант (A)	Число погибших животных				
		абс. (C)	% (M±m)		P
PLP = ФЛП	20	8	38,0±11,15	5,14	<1·10⁻²
Control = Контроль	20	21	62,0±10,66	2,6	<2·10⁻²
PLP = ФЛП + VB-2 = ВБ-2	13	3	23,0±11,68		

A = Variant; B = Total number of animals; C = Number of perishing animals; D = Absolute number.

From the data of Table 1 we can conclude that the PLP preparation has a protective effect against ionizing radiation. The effect of the PLP is intensified when it is combined with the VB-2 preparation, which is confirmed by

statistical treatment; in the first case $p < 1 \cdot 10^{-3}$, and in the second, $p < 2 \cdot 10^{-3}$.

The data showed that the condition of the blood of the experimental animals was much better than that of the control group. The content of hemoglobin in the experimental rats 5 days after irradiation was 103-105% of the initial level, followed by an insignificant drop to 95-96%. By the 30th day following irradiation, the hemoglobin content had risen to 100-104% of the initial level.

Figure 1. Variations in hemogram of rats irradiated from a Co^{60} source (700 r dose) with combined protection (PLP + VB-2). Average data. a -- experiment, b -- control. 1 -- hemoglobin, 2 -- erythrocytes, 3 -- reticulocytes, 4 -- thrombocytes. A = % of initial level; B = Days after irradiation.

In the control rats, starting on the 5th day following irradiation, there was a gradual decline in the hemoglobin content in comparison to the initial level; it reached its lowest values (47%) on the 15th day after irradiation. By the 30th day the hemoglobin content for the control group was 56%. The number of erythrocytes in the blood of the control rats from the 5th day after irradiation dropped gradually, reaching a minimum (60% of initial level) on the 20th day after irradiation. In the group I rats, in all time intervals (starting from the 5th day) following irradiation, the increase in the number of erythrocytes in comparison with the control specimens was statistically evident (with the exception of the 30th day following irradiation).

With combined protection, the survival rate was higher; on the 10th, 15th, and 20th day after irradiation, regeneration was more intense than with the administration of PLP alone. The increase in the erythrocyte count, starting on the 5th day, is statistically evident in all time intervals following irradiation (see Figs 1 and 2 and Table 2).

It must be noted that the decrease in the absolute reticulocyte count in the experimental group came later than in the control group. 5 days following irradiation, both the control and experimental specimens exhibited a sharp drop in the absolute reticulocyte count; for the experimental group, the count was 17-25% of the initial count; the corresponding figure for the control group was 8.37%. The regeneration process in the experimental group began sooner and was much more intensive than in the control group. With combined protection, the regeneration on the 10th, 15th, and 20th days following irradiation was more intense than with PLP protection alone.

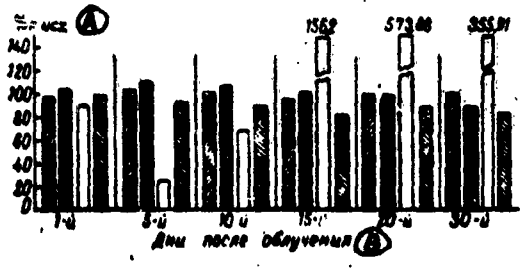


Figure 2. Variations in hemogram of rats irradiated from a Co^{60} source (700 r dose) with PLP protection. Average data. Notation same as in Fig 1. A = % of initial level; B = Days after irradiation.

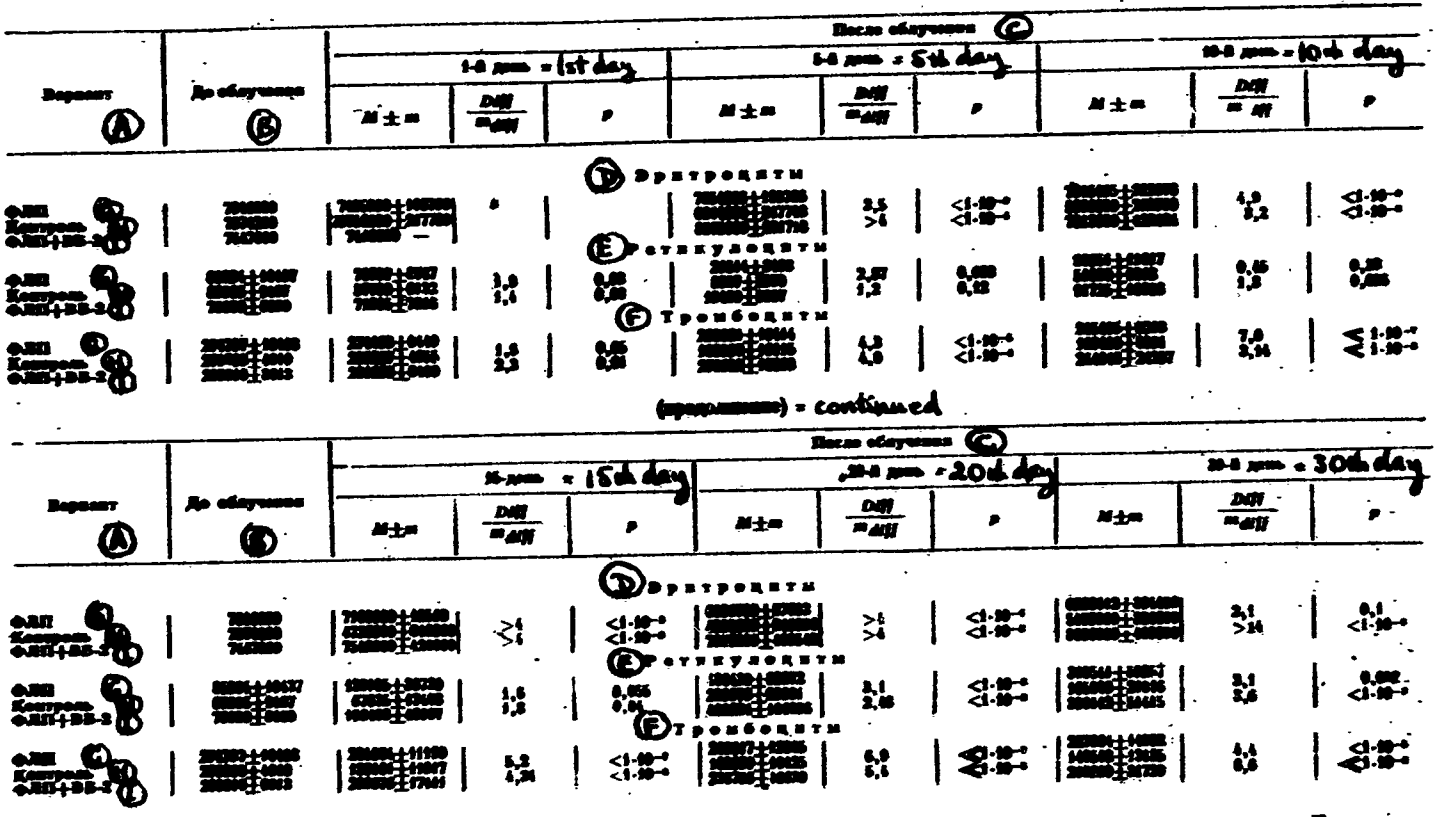
Fluctuations in the thrombocyte count over the entire observation period did not exceed normal limits. In the control group, the thrombocyte count from the 5th day following irradiation began to fall, reaching the lowest levels on the 15th, 20th, and 30th day following irradiation (see Table 2). The increase in the thrombocyte count in the experimental animals in all time intervals starting with the 5th day after irradiation was statistically evident in comparison with data for the control group.

With regard to the leucocyte count it must be noted that its increase became evident statistically just one day following irradiation.

The PLP preparation extracted from tea leaves, in contrast to BLP, does not produce a stable and clear varia-

Table 2

Changes in Hemograms of Experimental and Control Rats Irradiated From a Co^{60} Source (700 r dose)



A = Variant; B = Prior to irradiation; C = After irradiation; D = Erythrocytes; E = Reticulocytes; F = Thrombocytes; G = PLP; H = Control; I = PLP + VB-2.

tion in experiment in comparison with control in the picture of the leucocyte series.

Thus, we may conclude that the lipopolysaccharide preparation extracted from tea leaves has a protective action against radiation, increasing the survival rate of irradiated animals and producing an increase in the hemoglobin content and the erythrocyte, reticulocyte, and thrombocyte counts. The effect of this lipopolysaccharide preparation upon its combined use with VB-2 (the vinylbutyl ether polymer). As distinct from the bacterial lipopolysaccharides, the tea-leaf PLP has the ability to exercise a protective effect on the hemopoietic function and to stimulate erythro- and thrombocytosis without substantially and stably altering the picture of the leucocyte series.

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References

1. I. Fromme, O. Luederitz, et al., Pharmac. acta helv., 33, 391 (1958).
2. O. Westphal, Ann. Inst. Pasteur, 98, 789 (1960).
3. Z.V. Yermol'yeva, G.Ye., Vaysberg, et al., Antibiotics, No 6, 46 (1958).
4. H. Fricke, V. Probst, G. Schumacher, Schweiz. med. Wochensch., 88, No 28, 691 (1958).
5. J. Meyer-Kohn, A. Vogel, Arch. f. Klin. u. exp. Dermatol., 210, 31 (1960).
6. Ch. Borrel, J. Frei. A. Vanotti, Schweiz. med Wochenschr., 91, No 1, 8 (1961).
7. A.A. Yunis, W.J. Harrington, J. Lab. and Clin. Med., 55, No 4, 584 (1960).
8. J.G. Howard, J. Pathol. and Bacteriol., 78, No 2, 465 (1959).
9. A.I. Braude, G.Ye. Vaysberg, DAN (Doklady Akademii Nauk -- Reports of the Academy of Sciences USSR), 136, No 2 (1961).
10. B.N. Gladyshev, 5th International Biochemistry Congress, Section Report Abstracts, 2, 1961, p 100.
11. B.N. Gladyshev, DAN, 145, No 4 (1962).
12. V.A. Sondak, Ye. P. Gracheva, in the collection Vitaminnyye Resursy (Vitamin Resources), No 4, 1959, p 148.
13. R.A. Conard, Radiation Res., 5, 167 (1956).
14. R.A. Conard, J. Am. Physiol., 165, 375 (1951).

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ON THE SELECTIVE EFFECT OF ULTRASONIC WAVES ON THE MOLECULAR STRUCTURE OF INSULIN

[Following is the translation of an article by I. Ye. El'piner and L. I. Stekol'nikov, in the Russian-language periodical Doklady Akademii Nauk (Reports of the Academy of Sciences USSR), No 4, Moscow, 1962, pp 929-932.]

(Presented by Academician A.I. Oparin 2 July 1962)

In an ultrasonic field, proteins and enzymes split up into individual fragments of considerable molecular weight (refs 1 and 2). This process occurs only when the protein solution thus treated is saturated with oxygen (air) or argon and if the molecular weight of the protein is over 20,000. To start with, we had only indirect indications that upon the ultrasonic treatment of a protein solution in the presence of oxygen, it is possible to have not only the splitting of the protein molecules into relatively large molecular fragments, but also the removal from the protein molecule of individual aminoacids or minor peptides (refs 3, 4). Here in the case of insulin it was established that the hormonal function of this protein is intensified depending on the nature of the gas present in the treated solution (ref 3). The most obvious data on structural changes were obtained by us through the further study of uhf sound on water solutions of insulin.

The choice of insulin was dictated by the fact that the order of distribution of aminoacid residues in its molecule had been firmly established. The molecular weight of insulin is 6000 or 6000 X 2, which excludes the possibility of molecule splitting under the action of ultrasonic waves into relatively large fragments (ref 5). In addition, it is fairly easy to determine the biological activity of the hormone studied.

As is known, the insulin molecule consists of two polypeptide chains (A and B) connected by disulfide bridges (see diagram).

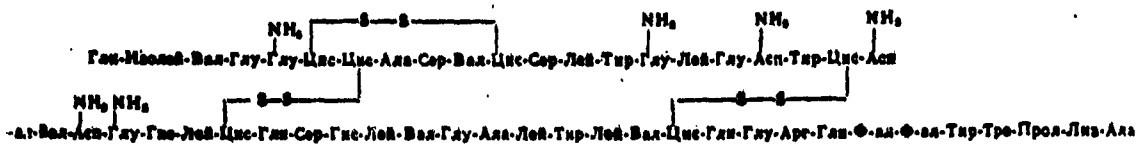
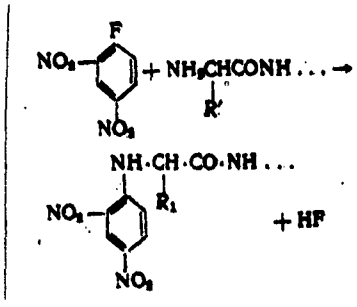


Diagram of Aminoacid Residue Distribution in Insulin Molecules [see English version p 9].

In the A-chain, the N-terminal bond aminoacid is glycine, and the C-terminal bond -- asparagine; in the B-chain, these are phenylalanine and alanine, respectively (refs 6, 7). To determine the N-terminal bond groups, we employed a method suggested by Sanger (refs 8, 9) based on the fact that dinitrofluorobenzol (DNFB) in a weakly-basic medium interacts with the free NH_2-groups of the protein to form dinitrophenyl derivatives:



The resultant product was subjected to acid hydrolysis in sealed ampules at 100° in 12 N HCl for 8 hours. Almost all of the dinitrophenol aminoacid derivatives (DNP-aminoacids) were extracted from the hydrolysate by means of ether. The free aminoacids were removed from the ether extract by solution in water, while the residual ether containing DNP-aminoacids was dissolved in alcohol and applied to chromatographic paper. The solvent employed was butanol saturated with 0.1% ammonia. The indicators were alcohol solutions of DNP-aminoacids.

The insulin we used, treated by the described method, produces two spots on the chromatogram (Fig 1) whose position coincides with the spots of the DNP-glycine and DNP-phenylalanine employed. These results confirm the data found in the literature which indicate that the N-terminal bond aminoacids are glycine and phenylalanine (refs 8-10) (the resulting dinitrophenol spot, a side reaction product, was brought out by placing the chromatogram into concentrated HCl vapor for 5-10 minutes).

Other results were obtained in the study of sound-treated insulin preparations. The treatment was applied to

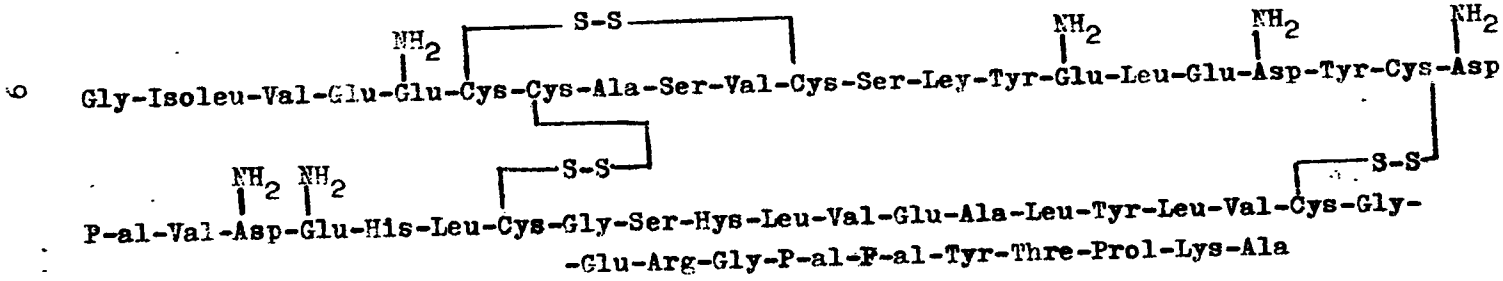


Diagram of Aminoacid Residue Distribution in Insulin Molecules

an 0.3% water solution of insulin for 3 hours with an ultrasonic wave intensity of about 10-12 watts/cm^2 (frequency -- 740 kilocycles/sec). The irradiated solution was saturated with oxygen, argon, or hydrogen.

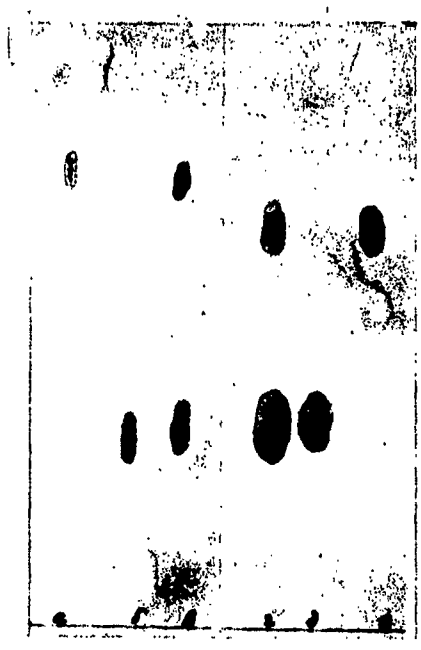


Fig 1

Fig 2

Figure 1. Chromatograms of N-terminal bond DNP-derivative aminoacids of insulin molecules. а -- DNP-phenylalanine; б -- DNP-glycine; в -- insulin not subjected to uhf sound treatment (lower spot -- DNP-glycine, upper spot -- DNP-phenylalanine); г -- insulin solution sound treated in presence of O_2 (lower spot -- DNP-glycine, upper spot -- DNP-histidine); д -- DNP-glycine; е -- DNP-histidine.

Figure 2. Chromatograms of N-terminal bond DNP-derivative insulin aminoacids for insulin subjected to uhf sound treatment in presence of argon. а -- DNP-phenylalanine + DNP-glycine; б -- DNP-alanine; в -- DNP-cystine; г -- insulin solution treated in presence of Ar (lower spot -- DNP-cystine or DNP-cysteine acid, upper spot -- DNP-phenylalanine).

Figure 1 is a chromatogram of an alcohol solution of DNP-derivative aminoacids obtained after appropriate hydrolysis of the insulin sound-treated in the presence of oxygen. The chromatograms invariably revealed a DNP-glycine and a DNP-

histidine spot. Consequently, under the action of uhf waves in the presence of oxygen, there is a cleavage of the peptide bond between the glutamine and histidine in the B chain. Further, the tetrapeptide P-al-Val-Asp(NH_2)-Glu(NH_2) breaks away from the insulin molecule. It should be noted that the chromatogram did not reveal a spot for DNP-phenylalanine which should have been formed as a result of DNFB interaction with phenylalanine which is the N-terminal bond aminoacid of the removed tetrapeptide. Apparently, the phenylalanine included in the tetrapeptide undergoes chemical transformations as a result of uhf sound irradiation which deprive it of the ability to react with DNFB.

It is interesting to note that in the case of insulin solution sound treatment in the presence of argon, it is the pentapeptide from the A chain of the hormone molecule rather than the tetrapeptide from the B chain which splits off. This is indicated by the chromatogram of Fig 2. Here the in-

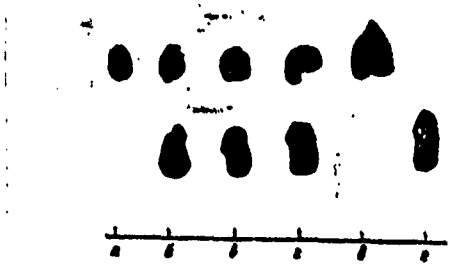


Figure 3. Chromatograms of aminoacids obtained as a result of the action of carboxypeptidase on insulin before and after its sound treatment. а -- untreated insulin; б -- insulin treated in presence of oxygen (upper spot -- alanine, lower spot -- aspartic acid); в -- insulin treated in presence of argon; г -- insulin treated in presence of H_2; д -- alanine; е -- aspartic acid.

sulin treated in the presence of argon and further processed with DNFB, produces two spots: DNP-phenylalanine and DNP-cystine. In other words, in the presence of argon under the action of uhf sound, there is a rupture of the polypeptide A chain with the appearance of cystine or its derivatives (DNP-cystine and its derivatives are close in their R_f) as the final N-aminoacid. In a number of cases, the chromatogram also revealed a glutamic acid spot. We used the dinitrofluorobenzol method unsuccessfully in an attempt to detect changes in the structure of insulin upon its sound treatment in the presence of hydrogen. Changes were detected, however, with another technique permitting the determination of the C-terminal bond aminoacids in the insulin. For this purpose

we used the carboxypeptidase method. Carboxypeptidase splits up largely those peptide bonds in the protein which are adjacent to the α-carboxyl group.

The insulin solution (50 mg of protein) (pH 7.8) in the presence of the carboxypeptidase enzyme was incubated at 37 and 25° for 4-6 hours. Trichloracetic acid (20%) was added following inactivation of the enzyme and the solution centrifuged. The fluid over the precipitate was decanted and evaporated in a vacuum (at 40°) until dry. The dry residue was dissolved in water and chromatographed (solvent -- butanol (CH_3COOH-H_2O); developer -- ninhydrine).

The untreated insulin produced a single spot on the chromatogram (Fig 3); this belonged to analine, as indicated in the literature (ref 11). From the same chromatogram we see that the preparations of insulin sound-treated in the presence of H_2, O_2, or Ar along with the alanine spot likewise yield an aspartic acid spot. This indicates that in the ultrasonic wave field at the end of the A-chain there takes place the deamidation of the aspartic acid which is split off from the protein by the carboxypeptidase. The deamidation of the asparagine (probably glutamine as well) was accompanied by the release of ammonia, which was detected by the Nessler reagent.

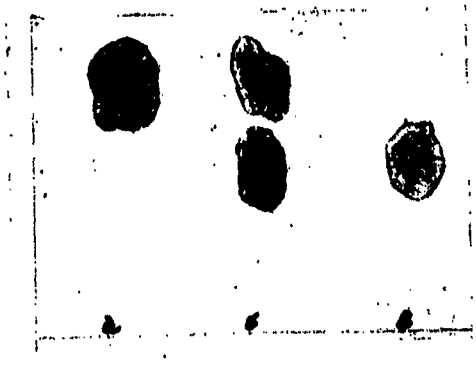


Figure 4. Chromatograms of asparagine before and after sound treatment in the presence of O_2 (asparagine concentration -- 10 mg/ml; length of treatment -- 10 hours; ultrasound frequency 10 watts/cm^2). а -- aspartic acid; б -- sound-treated asparagine (upper spot -- aspartic acid, lower spot -- asparagine); в -- asparagine.

It should be noted that asparagine under the action of uhf sound can actually undergo deamination, as indicated by the chromatogram (Fig 4).

It can also be surmised that the glutamine at the end of the polypeptide obtained in the sound treatment of the insulin solution undergoes deamination in the uhf wave field with the formation of glutamic acid (Fig 2).

Thus, uhf sound waves, depending on the nature of the gas present, produce ruptures in the polypeptide chain at strictly fixed points of the insulin molecule: in the presence of oxygen, a tetrapeptide splits off from the N-end of the B-chain; in the presence of argon, a pentapeptide splits off from the N-end of the A-chain. In the presence of hydrogen, we note only the deamination of asparagine on the C-end of the A-chain of the insulin molecule; the deamination of the asparagine is likewise observed with the sound treatment of insulin in the presence of O_2 and Ar. The latter fact indicates that asparagine deamination at the C-end of the insulin occurs as a result of both its interaction with O_2 and H_2 (activated in the ultrasound wave field), and as a result of its activation by OH-radicals whose formation is catalyzed in the presence of argon (ref 12).

The splitting off of the pentapeptide from the A-chain in the presence of argon with the participation of the OH-radicals occurs with the possible selective reaction of the latter with the aminoacid glutamine and cystine residues. The oxygen activated in the ultrasonic wave field apparently transforms chemically the aminoacid glutamine and histidine residues, as a result of which a tetrapeptide splits off from the B-chain of the insulin molecule.

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References

1. I.Ye. El'piner, G.A. Deborin, O.M. Zorina, Biokhimiya (Biochemistry), 24, 9117 (1959).
2. I.Ye. El'piner, Akusticheskiy Zhurnal (Journal of Acoustics), 6, 399 (1960).
3. I.Ye. El'piner, Byulleten' eksperimental'noy biologii i meditsyny (Journal of Experimental Biology and Medicine), 9, 207, 1950.
4. I.Ye. El;piner, O.M. Zorina, Biofizika (Biophysics), 5, 573 (1960).
5. G. Schmid, Zs. phys. Chem., 186A, 113 (1940).
6. F. Sanger, O. Thompson, Biochem J., 53, 353 (1953).
7. F. Sanger, H. Tuppy, ibid., 49, 463 (1951).
8. F. Sanger, ibid., 39, 507 (1945).
9. A. Ginsburg, H. Schachman, J. Biol. Chem., 235, 109 (1960).
10. F. Sanger, Biochem. J., 44, 126 (1949).
11. J. Lens, Biochim. biophys. acta, 3, 367 (1949).
12. I.Ye. El'piner, A.V. Sokol'skaya, DAN, 129, 202 (1959).

10,106
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